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A possible mechanism for the anxiolytic-like effect of gallic acid in the rat elevated plus maze

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ABSTRACT

This work was performed to characterize the possible mechanisms involved in the anxiolytic-like activity of gallic acid (GA) in the rat elevated plus maze (EPM) test. Male Wistar rats were acutely treated with a single dose of GA (10–500 mg/kg, i.p.) or diazepam and buspirone, 30 min prior to behavioral assessment in the EPM, open-field and rotarod tests. Treatment with GA markedly produced an increase in the time spent and entries in the open arms of EPM at doses of 30 and 300 mg/kg, respectively. These effects were comparable to those of the diazepam (1 mg/kg, i.p.) and buspirone (1 mg/kg, i.p.). Pretreatment with benzodiazepine antagonist flumazenil (3 mg/kg, i.p.) partially blocked the anxiolytic-like effect of GA. However, an increase in the time spent and entries in the open arms of EPM observed with GA treatment were significantly inhibited by the 5-HT_{1A} receptor antagonist WAY-100635 (0.5 mg/kg, i.p.). In the open-field test, only GA at a dose of 500 mg/kg decreased locomotor activity in rats. Moreover, GA (10–300 mg/kg, i.p.) or diazepam and buspirone did not alter motor coordination in the rotarod test. These results indicate that GA is an effective anxiolytic agent at low doses, while at the highest dose it has sedative effect. Also this study suggests that the anxiolytic-like activity of GA is primarily mediated by the 5-HT_{1A} but not benzodiazepine receptors.

1. Introduction

Anxiety, a state of excessive fear, is characterized by psychomotor tension, sympathetic hyperactivity, apprehension and vigilance syndromes (Sadock and Sadock, 2003). The benzodiazepines are considered as the main category of compounds prescribed for treatment of anxiety disorders. Unfortunately, they have several side effects such as tolerance, amnestic effect, etc. (Peng et al., 2004). The recognition of anxiolytic effects of non-benzodiazepine agents like buspirone that act *via* the serotonergic system and their beneficial effects in clinical anxiety and mood disorders has further focused attention on the 5-HT_{1A} receptors (Kunovac and Stahl, 1995). However, the anxiolytic effects of buspirone are delayed for 3–4 weeks, which is not like the fast effects observed with the benzodiazepines (Lowry et al., 2005). Thus, there is a need of robust anxiolytic compounds that have lesser side effects than benzodiazepines and a more immediate onset of action than the currently available 5-HT_{1A} receptor acting drugs. Especially, the use of mild, natural and tolerable compounds are in public favor for this purpose (Lake, 2000).

Flavonoids are naturally occurring polyphenolic compounds that have many biological properties, including antioxidative, anticancer, anti-inflammatory and neuroprotective effects. More recently, the actions of flavonoids on the central nervous system have attracted much attention. Additionally, flavonoids have demonstrated anxiolytic, sedative and anticonvulsant activities. Also, interactions of these compounds with the benzodiazapine binding sites of GABA-A receptors and adenosine receptors have been reported (Dekermendjian et al., 1999; Hanrahan et al., 2011; Medina et al., 1997).

Gallic acid (3,4,5-trihydroxybenzoic acid) and its derivatives are considered as the main polyphenolic compounds in grapes, different berries, mango, areca nut, walnut, green tea and other fruits as well as in wine (Singh et al., 2004). It has been reported to possess neuroprotective (Lu et al., 2006; Mansouri et al., 2013a,b; Reckziegel et al., 2011), cardioprotective (Hansi and Stanely, 2009), analgesic and anti-inflammatory (Kroes et al., 1992; Krogh et al., 2000), antihyperglycemic, antioxidant and an excellent free radical scavenging activities (Punithavathi et al., 2011). Toxicologic studies

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indicated that the acute toxicity of GA is very low; 4.1–4.6 g/kg (24–29 mmol/kg) subcutaneously or intraperitoneally is required for a 50% lethal dose to mice or rats (Shahrzad et al., 2001).

Growing evidences demonstrated that oxidative stress provoked anxious behavior in rodents (Rammal et al., 2008). Vignes et al. (2006) showed that (−)-epigallocatechin gallate (EGCG), a green tea polyphenol, exhibits dose-dependent anxiolytic, sedative-hypnotic and amnesiac activities, with evidence that these activities are mediated at least in part by $GABA_A$ receptors.

As mentioned above, GA has antioxidant and neuroprotective activities and also recently Dhingra et al. (2012) showed that repeated treatment of mice with GA produced anxiolytic-like effect in the elevated plus maze test (EPM) *via* nitriergic system. The aim of the present study was to assess the involvement of the GABA–benzodiazepine and 5-HT_{1A} receptors in the anxiolytic-like activities of single administration of GA in the rat EPM model.

2. Materials and methods

2.1. Animals and housing

Male Wistar rats weighing 180 ± 20 g were obtained from the animal facility of Ahvaz Jundishapur University of Medical Sciences (Ahvaz, Iran). Animals were kept five in each cage, in a temperature (22 ± 2 °C) and light (12-h light/dark cycle) controlled room, with free access to standard laboratory chow and water, *ad libitum*. All experiments were performed on separate groups of animals ($n = 10$) between 8 am and 1 pm, and each animal was used only once. The study was conducted in accordance with the NIH Guide for Care and Use of Laboratory Animals. The Institutional Animal Ethical Committee of Jundishapur University, formed under the Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA) approved the pharmacologic protocols.

2.2. Chemicals

Gallic acid HCl and WAY-100635 (5-HT_{1A} receptor antagonist, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]-ethyl]-*N*-(2-pyridinyl) cyclohexane carboxamide trihydrochloride) were obtained from Sigma (St. Louis, MO, USA). Flumazenil HCl (GABA–benzodiazepine antagonist) was purchased from Hoffmann-La Roche (Nutley, NJ, USA). Diazepam ampoules (10 mg/2 ml; Darupakhsh Pharmaceutical Co, Tehran, Iran) and buspirone HCl (Loghman Pharmaceutical Co, Tehran, Iran) were used as positive control drugs. Diazepam was diluted with deionized water containing 0.5% propylene glycol (Sigma-Aldrich, St. Louis, MO, USA). All other materials were of the highest grades commercially obtainable. All solutions were prepared freshly on test days and administered intraperitoneally (i.p.) in a volume of 4 ml/kg body weight of rats.

2.3. Elevated plus maze test

Anxiety was evaluated in the EPM test. The maze consisted of two open arms, 50 × 10 cm (length × width) and two closed arms, 50 × 10 × 50 cm (length × width × height) with an open roof arranged such that the two arms of each type were opposite to each other. The maze was elevated 50 cm above the ground and a video camera was fixed above the maze to record the movements for analysis. The behavioral experiments were conducted in a quiet room illuminated by a dim light (50 lx). For the test, each animal was placed in the center of the maze, facing one of the closed arms and the number of arms entries and the time spent on the open and closed arms were registered for 5 min by a trained observer (unaware of treatment assignment). The maze was cleaned with acetic acid 1.0% between animals to prevent olfactory cues from influencing the behavior of subsequently tested animals. The following measurements were taken and analyzed using the video-based Ethovision system (Nodulus, Wageningen, The Netherlands): the time spent in open arms was recorded and expressed as percentage of time in open arms, the number of entries into the open or closed arms (defined as placement of all four paws in the arms), and the total distance moved in the EPM (Pellow and File, 1986).

Animals received a single i.p. dose of saline, gallic acid, buspirone, or diazepam (1 mg/kg) and were tested for anxiolytic-like effect using the elevated plus maze. Gallic acid was dissolved in saline and given at five different dose ranges of 10, 30, 100, 300 and 500 mg/kg, in accordance with the results of a pilot study performed in our laboratory. The minimum dose that produced an increase in the time spent on the open arms by the animal was found to be 30 mg/kg, so we decided to increase the doses in a logarithmic fashion, i.e. 100 mg/kg and 300 mg/kg in order to find any dose–response relationship. Further, based on our preliminary results, one lower dose of gallic acid (10 mg/kg) was also included. Moreover, in rodents, the peak plasma concentration of gallic acid was reported to be achieved about 30 min after a single i.p. dose (Rasool et al., 2010; Shahrzad et al., 2001). Therefore, we have evaluated the anxiolytic-like activity 30 min after GA administration. Diazepam (1 mg/kg, single dose, i.p.; Bradley et al., 2007) and buspirone (1 mg/kg, single dose, i.p.; Kim et al., 2004) were also administered 30 min prior to testing as positive controls.

In a separate antagonism experiment, the rats were co-treated with GA, diazepam or buspirone and either flumazenil or WAY-100635. Fifteen minutes before administration of GA (30 or 300 mg/kg, i.p.), diazepam (1 mg/kg, i.p.), buspirone (1 mg/kg, i.p.) or their respective vehicles, animals were pretreated with flumazenil (3 mg/kg, i.p.) or WAY-100635 (0.5 mg/kg, i.p.) and were tested on the elevated plus maze after 30 min. The dose and pretreatment period of the two antagonists were chosen based on earlier reports and they showed neither anxiogenic property nor the effect on locomotion when administered alone (Girish et al., 2013; Grundmann et al., 2007; Seo et al., 2007; Yu et al., 2007). The treatment schedule and the intervals for estimation of various parameters have been presented in Fig. 1.

2.4. Spontaneous behavior in the open-field test

Spontaneous behavior in the open-field test was conducted in clear black Plexiglas boxes 40 × 40 × 40 cm (length × width × height) equipped with a video-based Ethovision system (Nodulus, Wageningen, The Netherlands) as described previously by Jung et al. (2006). The rats were placed in the center of the apparatus to assess locomotion (number of line crossing) and frequency of rearing or leaning 30 min after being treated with the single GA (10–500 mg/kg, i.p.), diazepam (1 mg/kg, i.p.), buspirone (1 mg/kg, i.p.) or vehicle (saline). The locomotor activity was recorded for 5 min.

2.5. Rotarod test

Rats were subjected to the rotarod test to evaluate the possible non-specific sedative or neuromuscular coordination effect. Rats were trained on the rotarod (Ugo Basile, Italy) accelerating from 0 to 40 revolutions/5 min, 3 times on the day before the experiment to allow accommodation to the testing apparatus. In the test day, animals were placed on the bar and then, the time each remained on the rotating rod was recorded (Dunham and Miya, 1957; Seo et al., 2007).

2.6. Statistical analysis

The obtained results were presented as means ± SEM. Data were evaluated by one-way analysis of variance (ANOVA) with Dunnett's tests for post hoc analysis. In the antagonism experiment, two-way ANOVA followed by the Bonferroni's test was used. All statistical analysis was done by using GraphPad Prism 5.0 (San Diego, CA, USA). Statistical significance was set at $P < 0.05$ (Sokal and Rohlf, 1981).